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(54) Title: TRANSGLUTAMINASE ENZYME INHIBITORS

(57) Abstract

The present invention relates to a composition and method for regulating protein crosslinking by transglutaminase enzymes in an animal or human. More particularly, the present invention relates to a series of novel transglutaminase enzyme inhibitors having the general formula: $R\text{-}C{=}O\text{-}(CH_2)_n\text{-}NCS$, wherein R is selected from the group consisting of $O\text{-}CH_3$, $O\text{-}CH_2\text{-}CH_3$, O-Ø, O-Bz, and NH_2 and n is an integer between 1 and 4, to their synthesis, and to their use in the treatment of crosslinking and amine incorporation disorders.

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TRANSGLUTAMINASE ENZYME INHIBITORS

Technical Field

The present invention relates to a series of novel transglutaminase enzyme inhibitors, to their synthesis, and to their use in the treatment of crosslinking and amine incorporation disorders. More particularly, the present invention relates to a composition and method for treating disease states involving altered activity of transglutaminase enzymes comprising using certain water stable transglutaminase enzyme inhibitors.

Background of the Invention

Transglutaminases (EC 2.3.2.13 - R-glutaminyl-peptide:-amine-γ-glutamyl-transferase) are Ca^{++}-dependent enzymes occurring widely in cells and body fluids. They catalyze nucleophilic displacement leading either to the cross-linking of proteins, creating γ-glutamyl-ε-lysine side chain peptides, or to the incorporation of small molecular weight amines into γ-glutamine sites of proteins. Transglutaminases contain an unusually reactive cysteine residue within their active site. This activated thiol reacts with the γ-carboxamide group of a protein bound glutamine residue, resulting in the formation of an activated thiol ester enzyme-substrate complex. The thiol ester intermediate is then subject to nucleophilic attack by a primary or secondary amine which results in a substituted γ-carboxamide group at the substrate glutamine residue and the regeneration of the active site thiol. Transglutaminases are extremely selective in

terms of the glutamine residues with which they will react. This selectivity depends on a combination of conformational parameters and on the amino acid sequence flanking the reactive glutamine. The hydrophobic binding pocket for the glutamine side chain assumes dimensions of between 4.5 and 5.5 Å by 2.5 Å. The enzyme sulfhydryl group is located at the apex of this pocket. Polypeptides containing a substrate glutamine residue bind in a single direction along the enzyme surface; the side chains of amino acids over a range of residues on each side of the substrate glutamine residue exert an influence on catalysis.

Calcium-dependent transglutaminases play an important role in the post-translational modification of proteins. Their activity has been implicated in the functioning of a number of physiological processes. These include, but are not limited to, inflammation, regulation of cell proliferation, metastatic potential of carcinomas, cell maturation, programmed cell death, epidermal differentiation, formation of epidermal cornified envelope, stabilization of hair, hemostasis, wound healing, receptor-mediated endocytosis, and receptor mediated phagocytosis in macrophages. Conversely, dysfunction of proteins catalyzed by abnormal transglutaminase activity are implicated in a number of pathologies including, but not limited to, inflammation, senile cataract formation, Alzheimer's disease, metastases, psoriasis, and secondary thrombosis.

The five transglutaminases about which most is known are listed in Table I. Each of these catalyze the crosslinking of proteins through amide bond formation between the carboxyl group of the γ-carbon of peptide-bound glutamine residues and the ε-amino group of peptide bound lysine residues. These ε-(γ-glutamyl)lysine isopeptide bonds are resistant to proteolysis, not readily degraded chemically, and they confer unique structure on their substrate proteins.

TABLE I

Summary of the Transglutaminases

Enzyme type	Source of Isolate	Distribution in tissue	MW KDa	Putative function
C	guinea pig liver	cytosolic enzyme	76.6	inflammation; programmed cell death
B	rat chondrosarcoma	particulate bound	95	protection of cancer cells against the lytic effects of autologous lymphokine activated killer cells
E	hair follicle		54	stabilization of the inner root sheath and medullary proteins of hair
	epidermis		77.8	formation of cornified epidermal envelope
K	human keratinocytes		92	formation of cornified epidermal envelope
Factor XIIIa	blood plasma	plasma, platelets and placenta	80.5	hemostasis

Transglutaminase C is a cytosolic enzyme found in endothelial and smooth muscle cells and is inducible in a number of other cells. It is present in most tissues and organs. Evidence that transglutaminase C crosslinking mediates phospholipase A_2 activation suggests a role for transglutaminase C in the regulation of the arachidonic acid cascade toward the leukotrienes and the resulting inflammation.

Programmed cell death involves the deletion of nucleated cells from living tissue. This occurs normally during embryonic development, red blood cell senescence, tissue turnover, hormone-induced atrophy, involution of hyperplasia, and regression of tumors. The activity of cytosolic transglutaminases are involved in these physiological processes. For examples, in erythrocytes, an increase in intracellular

calcium results in fusion between the cytoskeletal matrix and membrane proteins by ε-(γ-glutamyl)lysine bonds, giving rise to large immobile structures. This might be a general paradigm for cells undergoing terminal differentiation in the process of aging and dying. In fact, transglutaminase activity also has been implicated in at least two disease states related to cellular aging. These are senile cataract formation and Alzheimer's disease.

In senile cataract formation, the loss of transparency in the lens of the eye is related to a post-translational modification of the lens proteins themselves. This modification results from the crosslinking of the ß-crystallins through ε(γ-glutamyl)lysine bridges by the action of a transglutaminase similar to the cytosolic enzyme transglutaminase C.

Another area where transglutaminases may play a role is Alzheimer's disease. Alzheimer's disease is a selective neuronal degenerative disease which is identified by the presence of neuritic plaques and neurofibrillary tangles in the brain. Both of these pathoneurological structures contain abnormal paired helical intermediate filaments (PHF). The similarity of PHF to other proteins crosslinked by transglutaminases, the presence of factor XIIIa transglutaminase in brain, and the progressive accumulation of these structures in aging suggest the abnormal neuronal structure observed in Alzheimer's disease is a direct result of transglutaminase action on the amyloid proteins in the brain.

Other potential functions of cytosolic transglutaminases include, but are not restricted to, regulation of cell proliferation, receptor-mediated endocytosis, and receptor mediated phagocytosis in macrophages. Transglutaminases may also play a role in maintaining the integrity of the gastrointestinal, respiratory, and genitourinary tracts from developing localized immunological and/or inflammatory responses to foreign antigens. For example, there is evidence to suggest that transglutaminases play a role in masking the antigenicity of spermatozoa in the female genital tract and in developing

embryos during implantation. Moreover, it is possible that transglutaminase-dependent modification of protein structures may affect enzyme activity, protein turnover, and/or antigenicity resulting in autoimmunity.

Transglutaminase B, first isolated from rat swarm chondrosarcoma cells, is most abundant in tumor cells. It is postulated that, through the action of transglutaminase B, tumor cells coat themselves with fibrin thus masking recognition sites for immunocompetent cells. This suggests that the metastatic potential of carcinomas may be related to transglutaminase B activity.

Transglutaminase E is localized to the inner root sheath and medulla of the hair follicle where it functions to crosslink a citrulline-rich protein. The normal hair cycle in man and in some other mammals is characterized by periods of active growth followed by dormancy. This produces a continuous refurbishment without significant loss of hair in any specific area of the skin. An imbalance in the normal ordered array of hair structural proteins results in weakened structure, breaking of the hair shaft, and premature loss of hair.

Transglutaminase E and transglutaminase K both are found in epidermal tissue. They are an important marker for terminal epidermal differentiation and play a vital role in the ordered formation of normal cornified envelope. Epidermal transglutaminases are localized to the granular layer of the epidermis where they catalyze the crosslinking of a soluble cytoplasmic precursor to form the cornified envelope that lines the inner membrane of the mature keratinocyte in the stratum corneum, the visible layer of the epidermis. This layer forms an impervious barrier to external insult and confers stability, rigidity and elasticity on the skin.

Transglutaminase activity has been implicated in active psoriatic scale. In psoriatic scale, the normal kinetic constants of the membrane associated transglutaminase are significantly altered. Moreover, the crosslinking of the proteins

and the proteins themselves are different in active psoriatic scale from normal skin or from uninvolved psoriatic epidermis.

Plasma factor XIIIa is present in blood plasma, platelets, and the placenta. It crosslinks fibrin polymers in the progression from soft gel-like to hard rigid clots and is important in hemostasis and wound healing. Thrombosis results from arterial or venous occlusion by emboli. Proceeding from platelet aggregation intermeshed with fibrin polymer, the soft gel-like emboli are stabilized by the crosslinking of fibrin through the action of plasma factor XIIIa into a rigid clot. Thus, the action of plasma factor XIIIa may be considered an important determinant in thrombosis. Thrombi can cause blockage of blood vessels resulting in tissue damage and death. Dissolving thrombi after tissue damage has taken place has little effect as continuing therapy is needed even after restoration of blood flow and salvage of damaged tissue. Unfortunately, the anticoagulants currently used to prevent the formation of thrombi tend to reduce the body's capacity to stop bleeding or hemorrhage.

Control of enzyme activity is usually effected through the use of inhibitors. As the transglutaminases all act through a single cysteinyl moiety in the active site, the inhibitors used to date have been thiol-directed reagents acting through disulfide exchange or covalent bonding.

Among the disulfide exchange reagents, are tetrathionate and 5,5'-dithiobis(2-nitrobenzoic acid). These reagents have no specificity for transglutaminase, their effects are not active site directed, and they are of unknown toxicity.

Among the broad spectrum alkylators, the alpha-halo acetyl compounds bromoacetamide, chloroacetamide, and iodoacetamide show high reactivity. However, they are nonspecific as enzyme or protease inhibitors of transglutaminase activity. Alpha-bromo-4-hydroxy-3-nitroacetophenone shows the highest reactivity with the active site sulfhydryl of transglutaminase, but this reactivity is again nonspecific. L-N-(2-hydroxy-5-nitrophenylacetyl)-2-amino-4-oxo-5-chloropentanoate

may have specificity based upon its structural similarity to transglutaminase substrate z-glutamine-glycine. However, its properties *in vivo* are unknown. Glutamine-like analogs such as methyl N-(2-hydroxy-5-nitrophenylacetyl)-2-amino-4-oxo-5-chloropentanoate and 1-chloro-4-(2-hydroxy-5-nitrophenylacetyl)-amidobutan-2-one are more reactive than chloroacetamide, but less reactive than iodoacetamide. In general, the bromo-, chloro-, and iodo- alpha-halo ketones are too reactive to enable the high specificity needed for targeting drugs to a specific enzyme.

Other transglutaminase inhibitors have been evaluated. One compound, 2(1-acetonylthio)-5-methylthiazolo-(2,3,3)-1,3,4-thiadiazolium perchlorate, is reported to be a potent inhibitor of plasma factor XIIIa. Another compound, 3-Bromo-5-[N-carbobenzoxytyrosineamide(amino methyl)]-1,2-oxazoline, is reported to be a potent inhibitor of epidermal transglutaminase. However, limited information is available regarding the use of these drugs as transglutaminase inhibitors.

Both straight and branched chain isocyanates are rapid and effective inactivators of tissue transglutaminase when used at concentrations near that of the enzyme. Both the Ca^{++} dependent nature of the reaction and identification of the reactive group in the transglutaminase enzyme as a thiol provide evidence that isocyanates react with the active site cysteine sulfhydryl to inactivate transglutaminase. The linear structure of the isocyanate function satisfies the stringent annular requirement of the amide binding site of transglutaminase: the carboxamide group must fit within a site at the enzyme active center that has dimensions of between 4.5 and 5.5 Å by 2.5 Å. Use of the isocyanate function in a transglutaminase substrate carrier could be a potent specific inhibitor of the transglutaminases. However, such use, *in vivo*, is limited severely by extreme lability of isocyanates in water. For example, the half life of butyl isocyanate in water at 0°C and at pH 7.7 is approximately one minute.

Therefore, what is needed is a transglutaminase inhibitor that satisfies the stringent requirements of the amide binding site of the transglutaminase enzymes which is soluble in physiological solution. In addition, the selectivity of the transglutaminase cross-linking enzymes depends on a combination of conformational parameters and the amino acid sequence flanking the reactive glutamine. For specific and efficient inhibition of transglutaminase cross-linking enzyme activities, what is needed is an inhibitor that conforms both to the steric specificity of the hydrophobic binding pocket and to its surrounding structures.

Summary of the Invention

The present invention comprises a series of novel transglutaminase enzyme inhibitors, their synthesis, and their use in the treatment of transglutaminase enzyme dysfunction disorders. The transglutaminase dysfunction disorders are treated by administering an effective amount of an inhibitor to an animal or human in a pharmaceutically acceptable carrier.

The transglutaminase inhibitor of the present invention comprises the following general formula:

$$R\text{-}C{=}O\text{-}(CH_2)_n\text{-}NCS$$

wherein R is selected from the group consisting of O-CH_3, O-CH_2-CH_3, O-Ø, O-Bz, and NH_2 and n is an integer between 1 and 4.

Thus it is an object of the present invention to provide a composition which inhibits transglutaminase enzyme activities.

It is another object of the present invention to provide a composition which inhibits transglutaminase catalyzed incorporation of primary amines.

It is another object of the present invention to provide a composition which inhibits transglutaminase catalyzed cross-linking of proteins.

It is another object of the present invention to provide a composition which inhibits cytosolic transglutaminase enzyme activity.

It is another object of the present invention to provide a composition which inhibits liver transglutaminase enzyme activity.

It is another object of the present invention to provide a composition which inhibits particulate bound transglutaminase enzyme activity.

It is another object of the present invention to provide a composition which inhibits hair follicle transglutaminase enzyme activity.

It is another object of the present invention to provide a composition which inhibits epidermal transglutaminase enzyme activity.

It is another object of the present invention to provide a composition which inhibits blood plasma transglutaminase enzyme activity.

It is another object of the present invention to provide a composition and method for administering therapeutically effective doses of transglutaminase enzyme inhibitors in a pharmaceutically acceptable carrier.

It is another object of the present invention to provide a composition and method for the treatment of primary amine incorporation dysfunction disorders.

It is another object of the present invention to provide a composition and method for the treatment of crosslinking dysfunction disorders.

It is another object of the present invention to provide a composition and method for the treatment of active psoriatic scale.

It is another object of the present invention to provide a composition and method for the treatment of premature hair loss.

It is another object of the present invention to provide a composition and method for the treatment of metastatic carcinoma.

It is another object of the present invention to provide a composition and method for the treatment of inflammation.

It is another object of the present invention to provide a composition and method for the treatment of senile cataract formation.

It is another object of the present invention to provide a composition and method for the treatment of Alzheimer's disease.

It is another object of the present invention to provide a composition and method for the treatment of coagulation disorders.

It is another object of the present invention to provide a composition and method for prevention of thrombus formation.

It is another object of the present invention to provide a composition and method for reducing the need for anticoagulants in therapy of thrombosis and thereby lessen the danger of hemorrhage.

It is another object of the present invention to provide a composition and method for reducing the risk of rethrombosis after treatment with fibrinolytic enzymes.

It is another object of the present invention to provide a composition and method for reducing the risk of thrombosis immediately or at some time after invasive procedures which damage endothelial cells of the vasculature.

It is another object of the present invention to provide a composition that is stable in physiological fluids.

It is another object of the present invention to provide an economical process for the preparation of water soluble isothiocyanates in high yield.

These and other objects, features and advantages of the present invention will become apparent after a review of the following detailed description of the disclosed embodiments and the appended claims.

Detailed Description of the Disclosed Embodiments

In accordance with the present invention, a composition and method are provided for treating transglutaminase enzyme dysfunction disorders of humans and animals. The present invention includes a series of novel transglutaminase enzyme inhibiting compounds, their synthesis, and their use for treatment of disorders and conditions in which normal transglutaminase activity is pathologically increased. It is believed that this occurs in, but is not limited to, active psoriatic scale, metastases, senile cataract formation, Alzheimer's disease, and thrombus formation.

The method according to the present invention comprises administering to a animal or human suffering from a condition caused by a pathological increase in transglutaminase enzyme activity an effective amount of an isothiocyanate amide or ester. The isothiocyanate may be administered as a solution orally, intravenously or intramuscularly, or may be applied topically as a solution, cream or ointment by itself or with another agent or agents.

The transglutaminase inhibitor of the present invention comprises the following general formula:

$$R\text{-}C{=}O\text{-}(CH_2)_n\text{-}NCS$$

wherein R is selected from the group consisting of $O\text{-}CH_3$, $O\text{-}CH_2\text{-}CH_3$, O-Ø, O-Bz, and NH_2 and n is an integer between 1 and 4.

Not wanting to be bound by the following hypothesis, it is believed that the present invention acts according to the following mechanism: Inhibition of the transglutaminase enzymes is affected by reaction with the isothiocyanate functionality through formation of a dithiocarbamate with the active site cysteinyl sulfhydryl of the transglutaminase enzyme. The isothiocyanates possess a linear structure which satisfies the steric demands of the active site in the enzymes. Moreover, the isothiocyanates are stable in physiological solutions.

The transglutaminase enzyme inhibitors of the present invention can be administered by many different routes depending upon the particular symptom that is being treated. Administration routes include, but are not limited to, intravenous, intramuscular, subcutaneous, topical, intraperitoneal, and intranasal.

The transglutaminase enzyme inhibitors can be administered in a variety of pharmaceutically acceptable carriers including but not limited to oils, liquids, ointments, salves, and emollients.

General Synthetic Procedures

Compounds of the general structure:

$$R\text{-}C{=}O\text{-}(CH_2)_n\text{-}NCS$$

wherein R = OR' or NH_2

are prepared from their corresponding commercially available amino acid esters or from free amino acids via esterification and amidation to amino acid amides.

For example, the free amino acid is reacted with an appropriate alcohol, alkyl halide or other reagent known to those skilled in the art in the presence of a catalyst also known to those skilled in the art to form an ester.

$$NH_2\text{-}(CH_2)_n\text{-}C{=}O\text{-}OH \rightarrow NH_2\text{-}(CH_2)_n\text{-}C{=}O\text{-}OR$$

The ester is converted to an primary amide by procedures known to those skilled in the art. For example the ester is treated, under pressure, with an anhydrous saturated solution of ammonia in alcohol.

$$NH_2\text{-}(CH_2)_n\text{-}C{=}O\text{-}OR \rightarrow NH_2\text{-}(CH_2)_n\text{-}C{=}O\text{-}NH_2$$

The primary amine is reacted with reagents such as thiophosgene, thiocarbonyl dimidazole, or other reagents known to those skilled in the art to form an isothiocyanate.

$$NH_2\text{-}(CH_2)_n\text{-}C{=}O\text{-}NH_2 \rightarrow NCS\text{-}(CH_2)_n\text{-}C{=}O\text{-}NH_2$$

This reaction usually is carried out in a biphasic organic solvent-aqueous $NaHCO_3$ system. The organic solvent chosen is known by those skilled in the art to depend upon the solubility characteristics of the amino amides. The novel process of this invention for the preparation of isothicyanates comprises carrying out the reaction in total aqueous $NaHCO_3$. This greatly improves the yields of water soluble isothiocyanates obtained.

This invention is further illustrated by the following examples, which are not to be construed in any way as imposing limitations upon the scope thereof. On the contrary, it is to be clearly understood that resort may be had to various other embodiments, modifications, and equivalents thereof which, after reading the description herein, may suggest themselves to those skilled in the art without departing from the spirit of the present invention and/or the scope of the appended claims. Illustrative synthetic procedures which may be employed in the preparation of the isothiocyanate composition of the present invention include, but are not limited to, the following examples.

Example 1

γ-amino-n-butyric acid

NH_2-CH_2-CH_2-CH_2-C=OCH_3

A stream of hydrogen chloride gas is bubbled rapidly through 20 grams of γ-aminobutyric acid in 300 mL of absolute methanol until the γ-aminobutyric acid dissolves. The mixture is cooled to 0-5° C in an ice bath and the bubbling of hydrogen chloride gas is continued for an additional 30 minutes. The mixture is stirred overnight at room temperature and then concentrated to dryness under vacuum. The resultant white solid is suspended in 50 mL of methanol and again concentrated to dryness. This is repeated two additional times to remove excess hydrochloric acid. The white solid is recrystallized from methanol-ether to yield 24.19 g (176 mmoL) of γ-amino-n-butyric acid methyl ester-HCl.

Example 2

γ-amino-n-butyric acid amide

NH_2-CH_2-CH_2-CH_2-C=O-OCH_3 → NH_2-CH_2-CH_2-CH_2-C=O-NH_2

Ten grams of γ-aminobutyric acid methyl ester-HCl are added to 50 ml of a saturated solution of ammonia gas in methanol. The mixture is cooled to 0-5° C in an ice bath and a stream of ammonia gas is passed through the solution for 30 min. The solution is sealed in a pressure bottle, stirred for 2 days at room temperature, and concentrated under vacuum. The concentrate is diluted to 50 ml in methanol and concentrated again. This step is repeated several times. γ-amino-n-butyric acid amide hydrochloride is crystallized from the syrupy concentrate by the addition of methanol-ether.

Example 3

Isothiocyanatoacetamide

SCN-CH_2-C=O-NH_2

Isothiocyanatoacetamide is a novel water-soluble isothiocyanate compound which conforms to the stringent annular

requirement of the amide binding site of transglutaminase and its surrounding structures and is therefore a specific inhibitor of transglutaminase enzyme activity.

To prepare isothiocyanatoacetamide, freshly distilled thiophosgene (4.1 ml) is added dropwise to an ice-water cooled solution of 5.0 g of glycineamide hydrochloride in 200 ml of 1 M aqueous $NaHCO_3$ with constant stirring. The thiophosgene initially appears as orange droplets at the bottom of the reaction mixture, but as the reaction continues, the aqueous solution becomes orange. After 15 minutes, completion of the reaction is verified by thin layer chromatography (SiO_2, $CHCl_3$: CH_3OH: NH_4::6:4.5:0.05). The aqueous solution is extracted with ethyl acetate and the extract is washed twice with 10% HCl and once with brine. The solution is dried over anhydrous Na_2SO_4. Removal of the solvent under vacuum leaves an orange-brown solid which, following sublimation at 70° C under high vacuum, yields 2.7 g (51%) of isothiocyanatoacetamide as a white crystalline solid (mp 84-86° C).

Biological Evaluation

Inhibition Assay

The inhibitory potentials of the isothiocyanates are measured by their ability to inactivate purified preparations of the transglutaminases. Both a radioactive assay, which measures the incorporation of labelled putrescine into succinylated casein, and a colorimetric assay which measures the hydroxamate formed by hydroxylamine incorporated into Z-glutamylglycine, are used to measure transglutaminase enzyme activity. From these assays, percent inhibition is calculated from the amine-glutamine crosslink measured in the presence of isothiocyanate inhibitor, experimental sample, versus the amine-glutamine crosslink measured in the absence of isothiocyanate inhibitor, control sample. Percent inhibition, obtained using isothiocyanate inhibitors, is compared to that obtained using iodoacetamide - a known inhibitor of transglutaminase activity. (Folk, J.E. and S.I.

Chung in Methods in Enzymology, Meister A., ed., Vol. 112, pp. 358-361, Academic Press, New York 1985; Kim, H.C., M.S. Lewis, J.J. Gorman, S.C. Park, J. E. Girard, J.E. Folk, and S.I. Chung, J. BIOL. CHEM. Vol. 265, pp. 21971-21978, 1990).

Example 4

Inhibition of Liver Transglutaminase by Isothiocyanatoacetamide

Transglutaminase, purified from homogenized liver, is incubated with isothicyanatoacetamide for 15 min at 37°C and enzyme activity is measured in the colorimetric assay. Percent inhibition is calculated as the decrease in amine-glutamine crosslink in the experimental sample containing inhibitor compared to the control sample without inhibitor. The data summarized in Table A show that addition of isothiocyanatoacetamide to liver transglutaminase results in a loss of enzyme activity.

Table A

Molar inhibitor to enzyme ratios	% Inhibition
0.1	10%
0.5	50%
1	87%
10	100%
100	100%

These data suggest that the isothiocyanatoacetamide is reacting with the known active site sulfhydryl to inactivate the enzyme in a mole to mole manner. Under the same conditions, iodoacetamide also gives complete inactivation of the enzyme.

Example 5

Inhibition of Epidermal Transglutaminase by Isothiocyanato-acetamide

Transglutaminase, purified from epidermal tissue, is activated by Dispase for 15 minutes at 25°C. Enzyme activity is measured in the radioactive assay (Folk, J.E. and Chung, S.I. in Methods in Enzymology, Meister, A., ed., Vol. 112, pp. 358-361, Academic Press, New York, 1985) and percent inhibition is calculated as the decrease in incorporation of labelled putrescine into succinylated casein in the experimental sample, with inhibitor, compared to the control sample, without inhibitor. Addition of a 100-fold molar excess of isothiocyanatoacetamide to epidermal transglutaminase results in 45% inhibition of the enzyme. This inhibition is Ca^{++} dependent indicating that the inhibition is directed towards the active site cysteinyl residue. Under the same conditions, iodoacetamide gives 76% inhibition of the enzyme.

Example 6

Inhibition of Liver Transglutaminase by Isothiocyanatoacetamide and Alkyl Analogs

Transglutaminase, purified from liver, was incubated with isothiocyanatoacetamide, with alkyl isothiocyanates, and with iodoacetamide at 10 fold molar excess in 1% acetone-tris acetate buffer, pH 6, for 15 minutes at 37°C. Addition of isothiocyanatoacetamide and of a series of alkyl isothicyanates to transglutaminase isolated from liver results in a differential loss of liver transglutaminase enzyme activity. As shown in Table B, isothiocyanatoacetamide is from 13% to 41% more potent than the simple alkyl analogs as an inhibitor of the liver transglutaminase enzyme. As the degree of inhibition is a measure of specificity, the inclusion of the carbonyl moiety into an isothiocyanate inhibitor has increased the specificity of the compound.

Table B

Compound	% Inhibition
isothiocyanatoacetamide	90
iodoacetamide	99
methyl isothiocyanate	77
ethyl isothiocyanate	66
propyl isothiocyanate	62
butyl isothiocyanate	59
t-butyl isothiocyanate	49

Example 7

Comparison of the Inhibition of Liver Transglutaminase by Variable Chain Length Amide and Ester Isothiocyanates

Inhibition of the liver transglutaminase by 10 fold molar excess of variable chain length amide and ester isothiocyanates are summarized in Table C.

Table C

Compound	% Inhibition
isothiocyanatoacetamide	40
2-isothiocyanatoacetic acid ethyl ester	67
3-isothiocyanatopropionic acid ethyl ester	35
4-isothiocyanatobutyric acid ethyl ester	56
5-isothiocyanatopentanoic acid ethyl ester	64
iodoacetamide	97

The inhibitory potential of the carbonyl containing isothiocyanates shows that the optimal spacing of the isothiocyanate to carbonyl group is one or four methylene groups suggesting binding determinants in the active site α or δ to the isothiocyanate group.

It should be understood, of course, that the foregoing relates only to a preferred embodiment of the present invention and that numerous modifications or alterations may be made therein without departing from the spirit and the scope of the invention as set forth in the appended claims.

CLAIMS

I claim:

1. A composition for inhibiting transglutaminase enzyme activity comprising:

(a) an effective amount of a transglutaminase enzyme inhibiting compound with the following general formula:

$$R\text{-}C{=}O\text{-}(CH_2)_n\text{-}NCS$$

wherein R is selected from the group consisting of $O\text{-}CH_3$, $O\text{-}CH_2\text{-}CH_3$, O-Ø, O-Bz, and NH_2 and n is an integer between 1 and 4; and

(b) a pharmaceutically acceptable carrier.

2. The composition of Claim 1, wherein the transglutaminase enzyme activity is transglutaminase C activity.

3. The composition of Claim 1, wherein the transglutaminase enzyme activity is plasma factor XIIIa activity.

4. The composition of Claim 1, wherein the transglutaminase enzyme inhibiting compound is selected from the group consisting of isothiocyanatoacetamide, methyl isothyiocyanate, ethyl isothiocyanate, propyl isothiocyanate, butyl isothiocyante and t-butyl isothiocyanate.

5. The composition of Claim 4, wherein the transglutaminase enzyme inhibiting compound is isothiocyanatoacetamide

6. A method for inhibiting transglutaminase enzyme activity in an animal or human comprising the step of administering to the animal or human an effective amount of a transglutaminase enzyme inhibitor with the following general formula:

$$R\text{-}C{=}O\text{-}(CH_2)_n\text{-}NCS$$

wherein R is selected from the group consisting of $O\text{-}CH_3$, $O\text{-}CH_2\text{-}CH_3$, O-Ø, O-Bz, and NH_2 and n is an integer between 1 and 4.

7. The method of Claim 6, wherein the transglutaminase enzyme activity is transglutaminase C activity.

8. The method of Claim 6, wherein the transglutaminase enzyme activity is plasma factor XIIIa activity.

9. The method of Claim 6, wherein the transglutaminase enzyme inhibiting compound is selected from the group consisting of isothiocyanatoacetamide, methyl isothyiocyanate, ethyl isothiocyanate, propyl isothiocyanate, butyl isothiocyante and t-butyl isothiocyanate.

10. The method of Claim 9, wherein the transglutaminase enzyme inhibitor is isothiocyanatoacetamide.

11. A method of regulating protein crosslinking by transglutaminase enzymes in an animal or human comprising the step of administering to the animal or human an effective amount of a transglutaminase enzyme inhibitor with the following general formula:

$$R\text{-}C{=}O\text{-}(CH_2)_n\text{-}NCS$$

wherein R is selected from the group consisting of $O\text{-}CH_3$, $O\text{-}CH_2\text{-}CH_3$, O-Ø, O-Bz, and NH_2 and n is an integer between 1 and 4.

12. The method of Claim 11, wherein the transglutaminase enzyme activity is transglutaminase C activity.

13. The method of Claim 11, wherein the transglutaminase enzyme activity is plasma factor XIIIa activity.

14. The method of Claim 11, wherein the transglutaminase enzyme inhibiting compound is selected from the group consisting of isothiocyanatoacetamide, methyl isothyiocyanate, ethyl isothiocyanate, propyl isothiocyanate, butyl isothiocyante and t-butyl isothiocyanate.

15. The method of Claim 14, wherein the transglutaminase enzyme inhibitor is isothiocyanatoacetamide.

16. A method of treating tissue damage due to crosslinking dysfunctions in an animal or human comprising the step of administering to the animal or human an effective amount of a transglutaminase enzyme inhibitor with the following general formula:

$$R\text{-}C{=}O\text{-}(CH_2)_n\text{-}NCS$$

wherein R is selected from the group consisting of $O\text{-}CH_3$, $O\text{-}CH_2\text{-}CH_3$, O-Ø, O-Bz, and NH_2 and n is an integer between 1 and 4.

17. The method of Claim 16, wherein the transglutaminase enzyme activity is transglutaminase C activity.

18. The method of Claim 16, wherein the transglutaminase enzyme activity is plasma factor XIIIa activity.

19. The method of Claim 16, wherein the transglutaminase enzyme inhibiting compound is selected from the group consisting of isothiocyanatoacetamide, methyl isothyiocyanate, ethyl isothiocyanate, propyl isothiocyanate, butyl isothiocyante and t-butyl isothiocyanate.

20. The method of Claim 19, wherein the transglutaminase enzyme inhibitor is isothiocyanatoacetamide.

21. The method of Claim 16, wherein the tissue damage is epidermal damage.

22. The method of Claim 21, wherein the epidermal damage is active psoriatic scale.

23. The method of Claim 16, wherein the tissue damage is metastatic carcinoma.

24. The method of Claim 16, wherein the tissue damage is inflammation.

25. The method of Claim 16, wherein the tissue damage is senile cataract formation.

26. The method of Claim 16, wherein the tissue damage is neuronal degeneration.

27. The method of Claim 26, wherein the neuronal degeneration is Alzheimer's disease.

28. The method of Claim 16, wherein the tissue damage is the formation of thrombi.

29. The compound isothiocyanatoacetamide, having the chemical formula $SCN\text{-}CH_2\text{-}C{=}O\text{-}NH_2$.

30. A process for the preparation of the compound isothiocyanatoacetamide, comprising:

(a) adding distilled thiophosgene to an ice-water cooled solution of glycineamide hydrochloride in 1 M aqueous $NaHCO_3$ with constant stirring;

(b) extracting the aqueous solution with ethyl acetate;

(c) washing the extract with 10% HCl twice;

(d) washing the extract with brine once; and

(e) drying the extract over anhydrous Na_2S0_4.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US92/01180

I. CLASSIFICATI N F SUBJECT MATTER (if several classification symbols apply, indicate all)[3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5): A61K 31/13, A61K 31/16
US CL : 514/579

II. FIELDS SEARCHED

Minimum Documentation Searched[4]

Classification System	Classification Symbols
U.S.	514-579

Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched[5]

CAS ON LINE

III. DOCUMENTS CONSIDERED TO BE RELEVANT[14]

Category*	Citation of Document,[16] with indication, where appropriate, of the relevant passages[17]	Relevant to Claim No.[18]
A	JOURNAL OF BIOLOGICAL CHEMISTRY, VOL. 265, NO. 35, 17 MAY 1990, KIM ET AL. "PROTRANSGLJTAMINASE E FROM GUINEA PIG SKIN", PAGES 21971-21978.	1-30

* Special categories of cited documents:[15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search[2]	Date of Mailing of this International Search Report[2]
16 APRIL 1992	03 JUN 1992
International Searching Authority[1]	**Signature of Authorized Officer[20]**
ISA/US	FRIEDMAN/DC